09/593,288

Attachment #14

STN/East Search Strategy

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FILE 'MEDLINE' ENTERED AT 11:26:50 ON 18 SEP 2001

FILE 'CAPLUS' ENTERED AT 11:26:50 ON 18 SEP 2001
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FILE 'BIOSIS' ENTERED AT 11:26:50 ON 18 SEP 2001
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FILE 'AGRICOLA' ENTERED AT 11:26:50 ON 18 SEP 2001

=> s mitogen (s) activated (s) kinase (s) (mek6 or mkk6)
L1          237 MITOGEN (S) ACTIVATED (S) KINASE (S) (MEK6 OR MKK6)

=> s mitogen (2n) activated (2n) kinase
L2        34211 MITOGEN (2N) ACTIVATED (2N) KINASE

=> s l2 and (mek6 or mkk6)
L3          365 L2 AND (MEK6 OR MKK6)

=> s l3 and human
L4          185 L3 AND HUMAN

=> dup rem l4
PROCESSING COMPLETED FOR L4
L5           98 DUP REM L4 (87 DUPLICATES REMOVED)

=> s l5 and (histidine (3n) tag)
L6            0 L5 AND (HISTIDINE (3N) TAG)

=> s l5 and hemagglutinin
L7            0 L5 AND HEMAGGLUTININ

=> s l5 and glutathione
L8            4 L5 AND GLUTATHIONE

=> dup rem l8
PROCESSING COMPLETED FOR L8
L9            4 DUP REM L8 (0 DUPLICATES REMOVED)

=> d bib abs 1-4

L9   ANSWER 1 OF 4     MEDLINE
AN   2001290750     MEDLINE
DN   21269207   PubMed ID: 11238443
TI   Stress-induced inhibition of ERK1 and ERK2 by direct interaction with p38
     MAP kinase.
AU   Zhang H; Shi X; Hampong M; Blanis L; Pelech S
CS   Department of Medicine, Koerner Pavilion, University of British Columbia,
     Vancouver, British Columbia, Canada.
SO   JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Mar 9) 276 (10) 6905-8.
     Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY   United States
DT   Journal; Article; (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200107
ED   Entered STN: 20010723
     Last Updated on STN: 20010723
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Entered Medline: 20010719

AB We have identified a direct physical interaction between the stress signaling p38alpha MAP **kinase** and the **mitogen-activated** protein **kinases** ERK1 and ERK2 by affinity chromatography and coimmunoprecipitation studies. Phosphorylation and activation of p38alpha enhanced its interaction with ERK1/2, and this correlated with inhibition of ERK1/2 phosphotransferase activity. The loss of epidermal growth factor-induced activation and phosphorylation of ERK1/2 but not of their direct activator MEK1 in HeLa cells transfected with the p38alpha activator **MKK6**(E) indicated that activated p38alpha may sequester ERK1/2 and sterically block their phosphorylation by MEK1.

L9 ANSWER 2 OF 4 MEDLINE
AN 2001269993 MEDLINE
DN 21264827 PubMed ID: 11042204
TI p38 Kinase-dependent MAPKAPK-2 activation functions as 3-phosphoinositide-dependent kinase-2 for Akt in **human** neutrophils.
AU Rane M J; Coxon P Y; Powell D W; Webster R; Klein J B; Pierce W; Ping P; McLeish K R
CS Department of Medicine, University of Louisville Health Sciences Center and the Veterans Affairs Medical Center, Louisville, Kentucky 40202, USA..
mrane@louisville.edu
NC 1S10RR11368-01A1 (NCRR)
HL63901 (NHLBI)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Feb 2) 276 (5) 3517-23.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200106
ED Entered STN: 20010625
Last Updated on STN: 20010625
Entered Medline: 20010621

AB Akt activation requires phosphorylation of Thr(308) and Ser(473) by 3-phosphoinositide-dependent kinase-1 and 2 (PDK1 and PDK2), respectively. While PDK1 has been cloned and sequenced, PDK2 has yet to be identified. The present study shows that phosphatidylinositol 3-kinase-dependent p38 kinase activation regulates Akt phosphorylation and activity in **human** neutrophils. Inhibition of p38 kinase activity with SB203580 inhibited Akt Ser(473) phosphorylation following neutrophil stimulation with formyl-methionyl-leucyl-phenylalanine, FcgammaR cross-linking, or phosphatidylinositol 3,4,5-trisphosphate. Concentration inhibition studies showed that Ser(473) phosphorylation was inhibited by 0.3 &mgr;m SB203580, while inhibition of Thr(308) phosphorylation required 10 &mgr;m SB203580. Transient transfection of HEK293 cells with adenoviruses containing constitutively active MKK3 or **MKK6** resulted in activation of both p38 kinase and Akt. Immunoprecipitation and **glutathione** S-transferase (GST) pull-down studies showed that Akt was associated with p38 kinase, MK2, and Hsp27 in neutrophils, and Hsp27 dissociated from the complex upon activation. Active recombinant MK2 phosphorylated recombinant Akt and Akt in anti-Akt, anti-MK2, anti-p38, and anti-Hsp27

immunoprecipitates, and this was inhibited by an MK2 inhibitory peptide. We conclude that Akt exists in a signaling complex containing p38 kinase, MK2, and Hsp27 and that p38-dependent MK2 activation functions as PDK2 in **human** neutrophils.

L9 ANSWER 3 OF 4 MEDLINE
AN 2001284898 MEDLINE
DN 21068371 PubMed ID: 11156586
TI N-acetylcysteine attenuates TNF-alpha-induced p38 MAP kinase activation and p38 MAP kinase-mediated IL-8 production by **human** pulmonary vascular endothelial cells.
AU Hashimoto S; Gon Y; Matsumoto K; Takeshita I; Horie T
CS First Department of Internal Medicine, Nihon University School of Medicine, Tokyo 173-8610, Japan.. shuh@med.nihon-u.ac.jp
SO BRITISH JOURNAL OF PHARMACOLOGY, (2001 Jan) 132 (1) 270-6. Journal code: B00; 7502536. ISSN: 0007-1188.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200105
ED Entered STN: 20010529
Last Updated on STN: 20010529
Entered Medline: 20010524
AB 1. We have previously shown that tumour necrosis factor-alpha (TNF-alpha) activates p38 **mitogen-activated** protein (MAP) **kinase** to produce interleukin-8 (IL-8) by **human** pulmonary vascular endothelial cells. Reactive oxygen species (ROS) including H(2)O(2) generated by TNF-alpha can act as signalling intermediates for cytokine induction; therefore, scavenging ROS by anti-oxidants is important for the regulation of cytokine production. However, the effect of N-acetylcysteine (NAC), which acts as a precursor of **glutathione** (GSH) synthesis, on TNF-alpha-induced activation of p38 MAP kinase pathway and p38 MAP kinase-mediated IL-8 production by **human** pulmonary vascular endothelial cells has not been determined. To clarify these issues, we examined the effect of NAC on TNF-alpha-induced activation of p38 MAP kinase, MAP kinase kinase (MKK) 3 and **MKK6** which are upstream regulators of p38 MAP kinase, and p38 MAP kinase-mediated IL-8 production. 2. **Human** pulmonary vascular endothelial cells that had been preincubated with NAC were stimulated with TNF-alpha and then the activation of p38 MAP kinase and MKK3/**MKK6** in the cells and IL-8 concentrations in the culture supernatants were determined. 3. Intracellular GSH levels increased in NAC-treated cells. 4. NAC attenuated TNF-alpha-induced activation of p38 MAP kinase and MKK3/**MKK6**. 5. NAC attenuated p38 MAP kinase-mediated IL-8 production by TNF-alpha-stimulated cells. 6. These results indicate that the cellular reduction and oxidation (redox) regulated by intracellular GSH is critical for TNF-alpha-induced activation of p38 MAP kinase pathway and p38 MAP kinase-mediated IL-8 production by **human** pulmonary vascular endothelial cells, and we emphasize that anti-oxidant therapy is an important strategy for the treatment of acute lung injury.

L9 ANSWER 4 OF 4 MEDLINE
AN 96216353 MEDLINE
DN 96216353 PubMed ID: 8621675
TI Characterization of the structure and function of a novel MAP kinase kinase (**MKK6**).
AU Han J; Lee J D; Jiang Y; Li Z; Feng L; Ulevitch R J
CS Department of Immunology, The Scripps Research Institute, La Jolla,

California 92037, USA.
NC GM37696 (NIGMS)
GM51417 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Feb 9) 271 (6) 2886-91.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U39064; GENBANK-U39065; GENBANK-U39066
EM 199606
ED Entered STN: 19960627
Last Updated on STN: 20000303
Entered Medline: 19960619
AB **Mitogen-activated** protein (MAP) **kinases** require dual phosphorylation on threonine and tyrosine residues in order to gain enzymatic activity. This activation is carried out by a family of enzymes known as MAP kinase kinases (MKKs or MEKs). It appears that there are at least four subgroups in this family; MEK1/MEK2 subgroup that activates ERK1/ERK2, MEK5 that activates ERK5/BMK1, MKK3 that activates p38, and MKK4 that activates p38 and Jun kinase. Here we describe the characteristics of a new MKK termed **MKK6**. The clones we isolated encode two splice isoforms of **human MKK6** comprised of 278 and 334 amino acids, respectively, and one murine **MKK6** with 237 amino acids. Sequence information derived from cDNA cloning indicated that **MKK6** is most closely related to MKK3. The functional data revealed from co-transfection assays suggests that **MKK6**, like MKK3, selectively phosphorylates p38. Unlike the previously described MKKs (or MEKs), **MKK6** exists in a variety of alternatively spliced isoforms with distinct patterns of tissue expression. This suggests novel mechanisms regulating activation and/or function of various forms of **MKK6**.

	Type	L #	Hits	Search Text	DBs	Time Stamp
1	BRS	L1	355	mitogen-activated near2 kinase	USPAT; US-PG PUB; EPO; JPO; DERWENT; IBM	2001/09/18 11:15
2	BRS	L8	2	l1 and histidine-tag	USPAT; US-PG PUB; EPO; JPO; DERWENT; IBM	2001/09/18 11:12
3	BRS	L15	198	l1 and (GST or hemagglutinin-tag or tag)	USPAT; US-PG PUB; EPO; JPO; DERWENT; IBM	2001/09/18 11:13
4	BRS	L22	132	l1 and (GST or hemagglutinin-tag)	USPAT; US-PG PUB; EPO; JPO; DERWENT; IBM	2001/09/18 11:13
5	BRS	L29	0	l1 and hemagglutinin-tag	USPAT; US-PG PUB; EPO; JPO; DERWENT; IBM	2001/09/18 11:13

	Type	L #	Hits	Search Text	DBs	Time Stamp
6	BRS	L36	143	l1 and tag	USPAT; US-PG PUB; EPO; JPO; DERW ENT; IBM	2001/09/18 11:13
7	BRS	L43	46	l1 and hemagglutinin	USPAT; US-PG PUB; EPO; JPO; DERW ENT; IBM	2001/09/18 11:14
8	BRS	L50	40	l43 and tag	USPAT; US-PG PUB; EPO; JPO; DERW ENT; IBM	2001/09/18 11:14
9	BRS	L57	170	l1 and inactive	USPAT; US-PG PUB; EPO; JPO; DERW ENT; IBM	2001/09/18 11:15
10	BRS	L64	18	l57 and (MEK6 or MKK6)	USPAT; US-PG PUB; EPO; JPO; DERW ENT; IBM	2001/09/18 11:16